Differentiation Among Romanian Wine Regions Based on Lead Isotope Signatures

Florin Dumitru BORA[1], Anamaria CĂLUGĂR[2], Claudiu Ioan BUNEA[2,4*], Ionut RACZ[3]

[1] Department of Physico-Chemistry and Biochemistry, Research Station for Viticulture and Enology Târgu Bujoru, Romania
[2] Department of Horticulture and Landscaping, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[3] Wheat Breeding Department, Agricultural Research and Development Station Turda, Cluj-Napoca, Romania
[4] Department of Horticulture and Landscaping, Horticultural Research Station, Cluj-Napoca, Romania.
*corresponding author: claus_bunea@yahoo.com

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Abstract

The objective of this study is to determine the Pb concentration and $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{206}Pb/^{208}Pb$ from several Romanian winemaking regions, in order to highlight credible geographical markers of wine origin. The Pb level concentration and the ratios of $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{206}Pb/^{208}Pb$ were determined in 25 white and 9 red wines using ICP-MS techniques. Based on $^{206}Pb/^{207}Pb$ isotope ratios it can be concluded that the vines grown in the Tîrnave vineyard [Blaj (1.1790 average value)], Huși vineyard [Huși (1.1958 average value wine center), Averești (1.1908 average value)] and Iași vineyard [Copou (1.1875 average value) wine center], show traces of atmospheric pollution with lead [if $^{206}Pb/^{207}Pb = \sim 1.1700\text{-}1.2200$ (atmospheric pollution)]. Combining the $^{206}Pb/^{204}Pb$ with $^{206}Pb/^{208}Pb$ isotopic ratio may carry out a separation on the vineyards and wine-growing centers. Based on $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ a separation of the wine samples was possible.

Keywords: geographic origin, isotope ratio, wine fingerprint

Introduction

Among potentially toxic elements, Pb is possibly the most widely emitted by anthropogenic activities due to a long history of its utilization by mankind. The earliest known Pb artefact dates to 5600 BCE, and the processing to Pb minerals improved significantly about 6000 years ago (Nriagu, 1983). At present, Pb pollution has been reported world-wide (Martín *et al.*, 2014, Liu *et al.*, 2016). Lead contamination of soil poses potential health risks to humans via the food chain. Lead poisoning in adults can affect the nervous system, immune system, cardiovascular system and reproductive system (Needleman, 2004). Children are more susceptible to Pb than adults when exposed to Pb, which causes neuropsychological abnormalities and impaired learning abilities (Chiodo *et al.*, 2007). Therefore, prevention and control of Pb pollution are important environmental concerns (Liu *et al.*, 2019).

Source apportionment is necessary to determine control strategies to reduce Pb pollution and limit human exposure. Unravelling the relative contributions of Pb derived from different sources is the prerequisite for remediation (Morrison, 2000). Furthermore, information on source apportionment can help choose the appropriate remediation strategies. However, in most cases, the sources of Pb are complex, for example, soil Pb can originate from multiple sources and pathways, such as atmospheric deposition, irrigation, leaded pesticides, irrigation, and atmospheric deposition. The complexity of potential sources and the uncertainty of interactions among soil, water, air, and organisms may increase the difficulty of Pb source apportionment (Liu *et al.*, 2019).

The presence of Pb in wine is associated with two major sources as follows: human activity which results from the use of fertilizers, pesticides and agriculture, food additives, environmental pollution and natural sources which are due to the weathering of rocks (Almeida *et al.*, 2016).

In nature, there are three radioactive decay series, ^{232}Th, ^{238}U and ^{235}U. The parent element is a long-lived radioactive element and the last member of the series is a stable Pb isotope such as ^{206}Pb, ^{207}Pb or ^{208}Pb and is known as a radiogenic lead isotope. The stable Pb isotope, ^{204}Pb, is non-radiogenic. The Pb isotope ratio depends on factors such as U and Th content in the soil, weathering processes and original rock age, which provide a fingerprint used for different forensic and archeological purposes (Mihaljevič *et al.*, 2006).

The present study aimed to determine the Pb concentration and ^{206}Pb/^{204}Pb, ^{206}Pb/^{207}Pb, ^{206}Pb/^{208}Pb isotopic ratio from 25 white and 9 red wines using ICP-MS technique, in order to highlight credible geographical markers of wine origin. Wine samples were obtained under microvinification conditions of native and international varieties of vine.

Materials and methods

The samples used in this experiment were obtained from wines produced from Feteasca alba, Pinot gris, Sauvignon blanc, Feteasca regala, Italian Riesling, Muscat Ottonel, Feteasca neagra, Cabernet Sauvignon, Babeasca neagra, Sarba, Aligoté, Merlot and Busuioaca de Bohotin, under conditions of 2013-2017 from Tîrnave, Dealu Bujorului, Huși and Iași vineyards. The vineyards region is characterized by an alternate landscape, from flat to hilly areas, with altitude between 100 and 225 m and the predominant soil is levigated chernozem having a clayey sand texture with pH between values 7.4 and 8.1. Although they have moisture deficit, natural conditions (ecoclimatic and ecopedological) offer viable ecosystem for the development of vineyard. The wine samples were produced in micro-vinification conditions according to the methodology described by Bora *et al*. (2018). All vines were planted since 1979, and the vine plantation was organized with 2.2 × 1 m distance between rows and plants. Vines were pruned according to the Guyot system and were grown on speliers.

For the determination of Pb from wine samples were used in amount of 0.5 mL wine and adjust 8 mL (7 mL of 65% HNO_3 + 1 ml of 35% H_2O_2), after 15-45 minutes the mineralization was performed using Milestone START D Digestion System a microwave system. After mineralization process, wine samples were filtered through a 45.0 mm filter and brought to a volume of 50 ml. The method of microwave digestion was optimized in a previous work (Bora *et al.*, 2018).

The analysis was made using ICP-MS (iCAP Q Thermo Scientific) technique, after an appropriate dilution, using external standard calibration methods. The determination of Pb concentration and isotope ratio from wine was optimized in a previous work (Bora *et al.*, 2018). Isotopic reference materials of Pb (NIST SRM 981) from National Institute for Standards and Technology (NIST, Gaithersburg, MD, USA), were used for correction of the mass bias induced by instrumental mass discrimination. For determination of Pb in wine, calibration was performed using XXICertiPUR multielement standard.

For calibration and also to verify the achieved accuracy and precision, ten NIST-SRM 981 analysis results were pooled together with the calculated relative standard deviation presented in Table 1. Based on the obtained results, it was verified that applying quadrupole ICP-MS, relative standard deviation and reproducibility of approximately 0.5% for ^{87}Sr/^{86}Sr, ^{206}Pb/^{207}Pb and ^{208}Pb/^{206}Pb are feasible.

Results and discussions

The Pb concentration in the analysed wines corresponds to levels reported from neighboring countries and generally from the literature and in all exanimated samples was below the maximum admissible limit established by International Organization of Vine and Wine (O.I.V) reglemen-

Table 1. Lead isotopic ratio and Lead isotopic ratio determination precision and accuracy based on the NIST SRM 981 (Lead) (n=10)

Replicate	$^{207}Pb/^{206}Pb$ (a)	RSD (%)	$^{208}Pb/^{206}Pb$ (b)	RSD (%)	$^{204}Pb/^{206}Pb$ (c)	RSD (%)
1	0.46483	0.51	0.99891	0.67	0.00271	0.32
2	0.47891	0.48	0.99452	0.61	0.00272	0.41
3	0.46978	0.32	0.99794	0.55	0.00275	0.28
4	0.47123	0.64	0.99688	0.64	0.00273	0.51
5	0.46987	0.56	0.99726	0.48	0.00246	0.14
6	0.46154	0.37	0.99647	0.56	0.00258	0.39
7	0.47362	0.70	0.99969	0.34	0.00279	0.47
8	0.45641	0.43	0.99744	0.58	0.00278	0.51
9	0.41562	0.36	0.99576	0.59	0.00273	0.49
10	0.45612	0.45	0.99874	0.61	0.00278	0.36
Average	0.46179	0.48	0.99736	0.56	0.00270	0.41

(a) Certified value = $^{207}Pb/^{206}Pb$ (0.46707 ± 0.00020); (b) Certified value = $^{208}Pb/^{206}Pb$ (1.00016 ± 0.00036);
(c) Certified value = $^{204}Pb/^{206}Pb$ (0.027219 ± 0.00027); RSD (%) = relative standard deviation.

tation (150 µg/L) (O.I.V. 2016). However, significant differences can be observed among the values acquired for Pb in wines from Dealu Bujorului vineyard (70.61 µg/L), Huși vineyard (68.36 µg/L) and Averești wine center (66.64 µg/L), comparing to wines from Iași (48.96 µg/L) and Tîrnave (49.16 µg/L) vineyard. Early studies in England have reported Pb concentration as high as 1840 µg/L resulting from lad couldrons (Alkiș *et al.*, 2014). As stainless-steel boilers are used presently this problem has seemed to disappear.

The $^{206}Pb/^{207}Pb$ and $^{206}Pb/^{208}Pb$ ratios are commonly used as traces to differentiate natural and anthropogenic lead. In central Europe, the lead isotopic ratios, as signatures of pollution sources, ranges from relatively high $^{206}Pb/^{207}Pb$ ratios (fly ashes, coals and natural Pb, $^{206}Pb/^{207}Pb$ = ~ 1.1700 to 1.2200) to low $^{206}Pb/^{207}Pb$ values (petrol, gasoline, combustion, $^{206}Pb/^{207}Pb$ = ~ 1.0600 to 1.1400) (Avram *et al.*, 2014, Mihaljevič *et al.*, 2006, Bora *et al.*, 2018).

The values of $^{206}Pb/^{204}Pb$ and $^{206}Pb/^{208}Pb$ isotope ratios range from 16.3035 to 18.0325 ($^{206}Pb/^{204}Pb$) and 1.1950 to 2.3381 ($^{206}Pb/^{208}Pb$). The highest values of $^{206}Pb/^{204}Pb$ were registered in vine varieties grown in Tîrnave vineyard followed by vine cultivated in the vineyard of Dealu Bujorului. In $^{206}Pb/^{208}Pb$ the highest values were register to vine varieties grown in Tîrnave vineyard, while in the vine grown in Huși was recorded the lowest isotope ratio. The values of $^{206}Pb/^{204}Pb$ and $^{206}Pb/^{208}Pb$ isotope ratio obtained are comparable with Almedida *et al.* (2016) (2.0700 to 2.1570 Brazilian wines $^{206}Pb/^{208}Pb$, 16.6670 to 17.9960 Brazilian wines $^{206}Pb/^{204}Pb$) and also with Barbaste *et al.* (2001) (2.0990 to 2.1030 Italian wines $^{206}Pb/^{208}Pb$, 17.3210 Italian wines $^{206}Pb/^{204}Pb$).

Regarding $^{206}Pb/^{207}Pb$ isotope ratios based on our analyses it can be concluded that the vines grown in the Tîrnave vineyard [Blaj (1.1790 average value) wine center], Huși vineyard (1.1958 average value), Averești wine center (1.1908 average value) and Iași vineyard [Copou (1.1875 average value) wine center], the values of isotopic ratio of these varieties of vine show traces of atmospheric pollution with lead on vine (if $^{206}Pb/^{207}Pb$ = ~ 1.1700-1.2200 [atmospheric pollution]) (Tab. 2). The values of $^{206}Pb/^{207}Pb$ isotope ratios range between 1.1282 to 1.2642, values comparable with Avram *et al.* (2014) and Bora *et al.* (2018) (~1.1000 to 1.2000 Romanian wines). Regarding varieties of vine grown in Jidvei (1.1673) and Bujoru wine center (1.1557), the values of isotopic ratio of these vine varieties show traces indigenous lead ores (if $^{206}Pb/^{207}Pb$ = ~ 1.1500-1.1600 [indigenous lead ores]) (Lichfouse *et al.*, 2013).

The two-dimensional graphs (Fig. 1 and 2) and show that it is possible to differentiate even among the vineyards (Tîrnave, Dealu Bujorului, Huși and Iași) or wine centers (Blaj, Jidvei, Bujoru, Huși, Averești and Copou) based on the $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{204}Pb$ isotopic ratios.

Combining the $^{206}Pb/^{204}Pb$ with $^{206}Pb/^{208}Pb$ isotopic ratio may carry out a separation on the samples of wine analyzed on the basis of these isotopic ratios. Wines were grouped in their

Table 2. The content of Pb (μg L^{-1}) and $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{206}Pb/^{208}Pb$ from wine samples

Vineyard	Wine Center	Variety	Pb μg L^{-1} ± SD (M.L.A. 150 μg/L)	$^{206}Pb/^{204}Pb$	RSD / %	$^{206}Pb/^{207}Pb$	RSD / %	$^{206}Pb/^{208}Pb$	RSD / %
Tîrnave	Blaj	Feteasca alba	30.52 ± 2.11 $^{kl\ \gamma}$	16.8883 $^{de\ \alpha}$	0.7	1.1703 $^{bc\ \alpha}$	0.1	2.2448 $^{abcdefg\ \alpha}$	0.1
		Pinot gris	46.06 ± 8.40 $^{defgh\ \alpha}$	16.7396 $^{defg\ \alpha}$	0.5	1.1956 $^{abc\ \alpha}$	0.1	2.2453 $^{abcdefg\ \alpha}$	0.9
		Sauvignon blanc	20.18 ± 4.43 $^{mn\ \delta}$	16.5684 $^{efgh\ \alpha}$	0.5	1.1812 $^{bc\ \alpha}$	0.9	2.2392 $^{abcdefg\ \alpha}$	1.8
		Feteasca regala	35.86 ± 5.27 $^{ijk\ \beta\gamma}$	16.4355 $^{gh\ \alpha}$	0.2	1.1938 $^{abc\ \alpha}$	0.5	2.3036 $^{ab\ \alpha}$	0.7
		Italian Riesling	20.72 ± 1.80 $^{mn\ \delta}$	16.7347 $^{defg\ \alpha}$	0.3	1.1992 $^{abc\ \alpha}$	1.3	2.3381 $^{a\ \alpha}$	0.3
		Muscat Ottonel	42.89 ± 5.58 $^{efghi\ \alpha\beta}$	16.8200 $^{def\ \alpha}$	0.1	1.1638 $^{bc\ \alpha}$	0.8	2.3103 $^{ab\ \alpha}$	05
	Jidvei	Feteasca alba	32.33 ± 5.90 $^{jkl\ \beta}$	16.5649 $^{efgh\ \alpha}$	0.5	1.1712 $^{bc\ \alpha}$	0.6	2.2575 $^{abcdef\ \alpha}$	0.6
		Pinot gris	46.09 ± 5.96 $^{defgh\ \alpha}$	16.4267 $^{gh\ \alpha}$	0.8	1.1648 $^{bc\ \alpha}$	0.4	2.2980 $^{abc\ \alpha}$	0.1
		Sauvignon blanc	21.01 ± 1.28 $^{mn\ \gamma}$	16.4675 $^{fgh\ \alpha}$	0.4	1.1555 bc $^{\alpha}$	0.1	2.2846 $^{abcd\ \alpha}$	0.4
		Feteasca regala	49.16 ± 2.65 $^{defg\ \alpha}$	16.3035 $^{h\ \alpha}$	0.2	1.1688 $^{bc\ \alpha}$	1.3	2.2673 $^{abcde\ \alpha}$	0.7
		Muscat Ottonel	42.54 ± 1.37 $^{fghi\ \alpha}$	16.4344 $^{gh\ \alpha}$	0.8	1.1762 $^{bc\ \alpha}$	0.8	2.2339 $^{abcdefg\ \alpha}$	1.6
Dealu Bujorului	Bujoru	Feteasca alba	23.56 ± 2.62 $^{lm\ \delta}$	16.6558 $^{efgh\ \alpha\beta}$	0.3	1.1337 $^{bc\ \beta}$	1.9	2.1251 $^{fghijk\ \alpha}$	0.1
		Feteasca regala	24.30 ± 1.22 $^{lm\ \delta}$	16.6295 $^{efgh\ \alpha\beta}$	0.4	1.1352 $^{bc\ \beta}$	0.3	2.1661 $^{cdefghij\ \alpha}$	0.8
		Feteasca neagra	70.61 ± 7.37 $^{a\ \alpha}$	16.6194 $^{efgh\ \alpha\beta}$	0.9	1.1347 $^{bc\ \beta}$	0.6	2.1534 $^{defghijk\ \alpha}$	0.6
		Cabernet Sauvignon	53.93 ± 2.44 $^{cd\ \beta}$	16.6508 $^{efgh\ \alpha\beta}$	0.2	1.1378 $^{bc\ \beta}$	2.5	2.1321 $^{fghijk\ \alpha}$	0.4
		Babeasca neagra	38.69 ± 3.09 $^{hijk\ \gamma}$	16.6637 $^{efgh\ \alpha\beta}$	0.5	1.1282 $^{c\ \beta}$	1.2	2.1871 $^{bcdefghi\ \alpha}$	0.2
		Sarba	53.30 ± 8.36 $^{cd\ \beta}$	16.5457 $^{efgh\ \beta}$	0.1	1.2642 $^{a\ \alpha}$	0.3	2.1489 $^{efghijk\ \alpha}$	0.7
		Muscat Ottonel	13.88 ± 3.20 $^{n\ \varepsilon}$	16.7160 $^{defg\ \alpha}$	0.7	1.1563 $^{bc\ \beta}$	0.6	2.1858 $^{bcdefghi\ \alpha}$	0.5
Huși	Huși	Aligoté	68.36 ± 4.19 $^{ab\ \alpha}$	17.3225 $^{bc\ \alpha\beta}$	0.4	1.2010 $^{abc\ \alpha\beta}$	0.5	2.0198 $^{k\ \beta}$	0.3
		Feteasca regala	47.56 ± 3.27 $^{defgh\ \beta}$	17.3989 $^{b\ \alpha}$	0.7	1.2097 $^{ab\ \alpha}$	0.9	2.0417 $^{jk\ \beta}$	0.9
		Feteasca alba	30.20 ± 1.75 $^{kl\ \delta\varepsilon}$	17.3556 $^{bc\ \alpha}$	1.3	1.2013 $^{abc\ \alpha\beta}$	0.1	2.0625 $^{ijk\ \beta}$	0.2
		Italian Riesling	35.57 ± 5.48 $^{ijk\ \gamma\delta}$	17.3231 $^{bc\ \alpha\beta}$	0.5	1.1988 $^{abc\ \alpha\beta}$	0.2	2.0531 $^{ijk\ \beta}$	0.1
		Babeasca neagra	52.08 ± 2.74 $^{cde\ \beta}$	17.4117 $^{b\ \alpha}$	0.4	1.2114 $^{ab\ \alpha}$	0.5	2.0797 $^{hijk\ \alpha\beta}$	0.5
		Feteasca neagra	38.97 ± 2.56 $^{hijk\ \gamma}$	17.3567 $^{bc\ \alpha}$	1.9	1.1994 $^{abc\ \alpha\beta}$	0.3	2.0813 $^{hijk\ \alpha\beta}$	0.9
		Cabernet Sauvignon	45.76 ± 6.35 $^{defgh\ \beta}$	17.4959 $^{b\ \alpha}$	0.2	1.1935 $^{abc\ \alpha\beta}$	0.2	2.0632 $^{ijk\ \beta}$	0.6
		Merlot	25.11 ± 1.22 $^{lm\ \varepsilon}$	17.0323 $^{cd\ \beta}$	0.5	1.1759 $^{bc\ \alpha\beta}$	0.6	2.1189 $^{ghijk\ \alpha\beta}$	0.8
		Busuioaca de Bohotin	52.19 ± 0.95 $^{cde\ \beta}$	17.4185 $^{b\ \alpha}$	0.1	1.1709 $^{bc\ \beta}$	0.7	2.2007 $^{bcdefgh\ \alpha}$	0.6
	Averești	Feteasca alba	66.64 ± 4.28 $^{ab\ \alpha}$	17.3667 $^{bc\ \alpha}$	0.9	1.1869 $^{bc\ \alpha}$	2.8	2.0953 $^{hijk\ \alpha}$	0.1
		Feteasca regala	51.46 ± 3.61 $^{cdef\ \beta}$	17.5741 $^{b\ \alpha}$	0.6	1.1810 $^{bc\ \alpha}$	0.3	2.0907 $^{hijk\ \alpha}$	0.2
		Feteasca neagra	60.32 ± 4.21 $^{bc\ \alpha\beta}$	17.4140 $^{b\ \alpha}$	1.6	1.2003 $^{abc\ \alpha}$	0.3	2.0673 $^{hijk\ \beta}$	0.2
		Merlot	65.06 ± 6.33 $^{ab\ \alpha}$	17.42696 $^{b\ \alpha}$	0.2	1.1950 $^{abc\ \alpha}$	0.1	1.1950 $^{bcdefgh\ \alpha}$	0.6
Iași	Copou	Feteasca regala	48.96 ± 11.77 $^{defg\ \alpha}$	18.0652 $^{a\ \alpha}$	0.6	1.1759 $^{bc\ \alpha}$	0.8	2.1673 $^{cdefghij\ \alpha}$	0.2
		Feteasca alba	40.10 ± 3.51 $^{ghij\ \alpha}$	18.0252 $^{a\ \alpha}$	0.9	1.1943 $^{abc\ \alpha}$	0.5	2.1286 $^{fghijk\ \alpha}$	0.1
		Italian Riesling	48.33 ± 6.95 $^{defg\ \alpha}$	18.0325 $^{a\ \alpha}$	0.8	1.1925 $^{abc\ \alpha}$	0.2	2.1455 $^{efghijk\ \alpha}$	0.5

Average value ± standard deviation (n = 3). Roman letters represent the significance of grape varieties cultivated in different wine center (Duncan test, $p < 0.05$). Greek letters represent the significance of grape varieties cultivated in the same wine center (Duncan test, $p < 0.05$). The difference between any two values, followed by at least one common leter, is insignificant. M.P.L = maximul permissible allowed (O.I.V., 2016). LOQ = lower that the limit of quantification.

distinct groups corresponding to vineyard of provenance (Fig. 3).

Variation of $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ in wines with different geographical origins confirm the link with geological substratum of the production territory, making the $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{206}Pb/^{208}Pb$ a robust instrument for tracing the geographical provenance of wines.

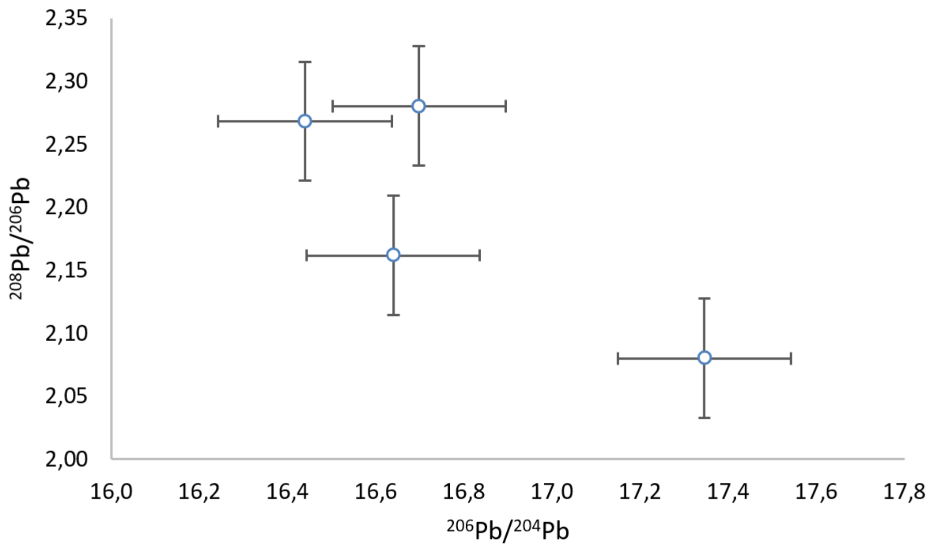

Figure 1. Distributions of wine samples based on the isotopic ratios of the vineyard area

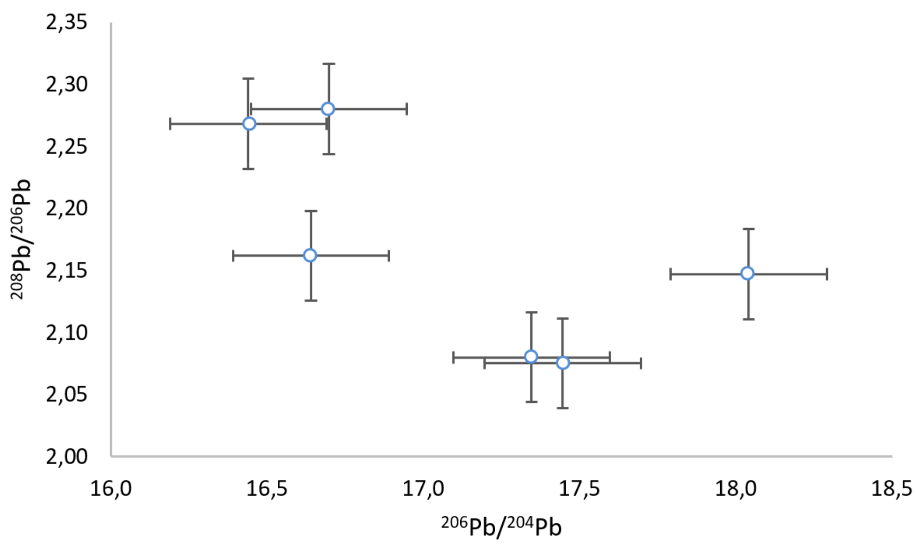

Figure 2. Distributions of wine samples based on the isotopic ratios of the wine center area

Conclusion

Pb concentration found in wine from four vineyards studied did not vary greatly and was well below the limits established by Organization of Vine and Wine. However, significant differences can be observed among the values acquired for Pb in wines from Dealu Bujorului and Huși, comparing to wines from Iași and Tîrnave vineyards. Studies involving isotopic ratios of lead have shown promise in differentiating the wine producing regions of Romania. Regarding $^{206}Pb/^{207}Pb$ isotope ratios based on analyses it can be concluded that the vines grown in the Tîrnave vineyard [Blaj wine center (1.1790 average value)], Huși vineyard [Huși (1.1958 average value), Averești wine center (1.1908 average value)] and Iași vineyard [Copou wine center (1.1875 average value)], the values of isotopic ratio of these varieties of vine show traces of atmospheric pollution with lead on vine (if $^{206}Pb/^{207}Pb = \sim 1.1700\text{-}1.2200$ [atmospheric pollution]). Combining the $^{206}Pb/^{204}Pb$ with $^{206}Pb/^{208}Pb$ isotopic ratio may carry out a separation on the vineyards and wine-growing centers. Based on $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ a separation of the wine samples was possible.

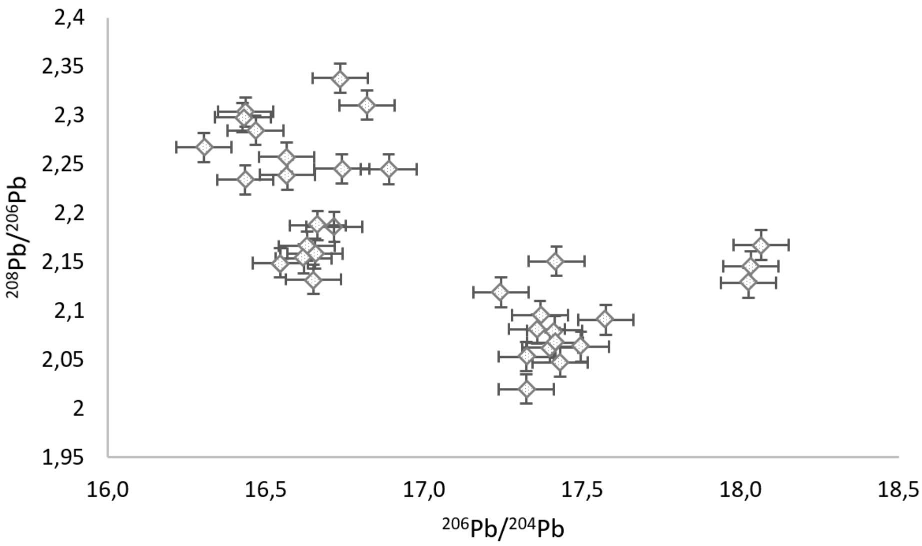

Figure 3. Distributions of wine samples based on the isotopic ratios

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